Amendments to Detailed Description

A number of SEQ ID NOs are entered where they originally appeared as "SEQ ID NO: __" on page 39 of the specification. The entered SEQ ID NOs and the corresponding sequences were already disclosed in the sequence listing submitted July 8, 1999, as amended to comply with C.F.R. §1.822 on February 25, 2000. In addition, certain SEQ ID NO designations as they appear on page 39 of the specification are amended to correspond to the existing sequence listing. These include SEQ ID NOs: 50, 56, and 60, now amended to be designated as SEQ ID NOs: 59, 97, and 103, respectively. No change to the sequences themselves is made. A new sequence listing and CRF are therefore not required.

Amendment of Claims 10, 12, 39, 41, 48, 50, 61, and 63

Claims 10, 12, 39, 41, 48, 50, 61, and 63 are amended to substitute the conjunction "and" for "or" in the Markush group describing the nucleotides represented by X_3X_4. This amendment merely places each affected Markush group in standard Markush form. MPEP 2173.05(h).

Amendment of Claims 1, 6, 7, 10, 11, 27-30, 35, 36, 39, 40, 42, 44, 45, 48, 49, 51-54, 57, 58, 61, and 62

Each of these claims has been amended to replace the term "oligonucleotide" or "oligonucleotide having a sequence including . . ." with the term CpG oligonucleotide. The term "CpG oligonucleotide" clearly and concisely defines the scope of the compound causing the immune response. The term CpG oligonucleotide is used throughout the application, including the background, summary, and detailed description sections of the application. The term is also used consistently with its well established meaning in art. As set forth in the background section, this class of molecules having a CG dinucleotide with an unmethylated C is well known. It was established prior to the instant invention that nucleic acids containing an unmethylated CG dinucleotide stimulate an immune response, e.g., B cell activation, but that this immune stimulating capability is abolished when the C is methylated. Thus, the class of compounds referred to as "CpG oligonucleotides" is well known.

Claims 11, 40 ,49, and 62 also have been amended to correct typographical errors involving subscripts and duplicate punctuation, so that in each of these claims the phrase "X_1, X_2, X_3,, and X_4" now correctly reads "X_1, X_2, X_3, and X_4".

New Claims 66-77

New independent claim 66 is directed to a method for inducing an antigen-specific immune response in a nonhuman vertebrate, involving administering to the nonhuman vertebrate a CpG oligonucleotide, wherein the oligonucleotide includes at least 8 nucleotides, and exposing the nonhuman vertebrate to an antigen at least 3 days after the oligonucleotide is administered to the nonhuman vertebrate to produce an antigen-specific immune response. Support for this claim can be found in the specification at page 16, line 23 through page 17, line 7; page 21, lines 15-23; page 40, lines 10-11; page 64, lines 8-18; and Figure 17.

Claims 67 and 68 depend from claim 66 and specify narrower specific formulas of the CpG oligonucleotides. Support for these claims is found throughout the specification and in original claims 10 and 12.

Claim 69 depends from claim 66 and specifies certain nonhuman vertebrates to include dog, cat, horse, cow, pig, sheep, goat, chicken, monkey, and fish. Support for this claim can be found on page 21, lines 20-21.

Claims 70 and 71 also depend from claim 66 and specify that the antigen is administered at least 15 days (claim 70) or at least 30 days (claim 71) after the oligonucleotide is administered. Support for these claims can be found on page 21, lines 15-18.

New claim 72 depends from claim 66 and specifies the source from which the antigen is derived. Support for this claim is found at least on page 17-20 and 21-30.

New independent claim 73 is directed to a method for increasing platelet counts in a nonhuman vertebrate having thrombocytopenia, in which a nonhuman vertebrate having thrombocytopenia is administered a CpG oligonucleotide, wherein the oligonucleotide includes at least 8 nucleotides, in an amount effective to increase platelet counts in the nonhuman vertebrate. Support for this claim can be found in the specification at page 44, lines 6-12; and page 21, lines 19-21.

Claim 74, which depends from claim 73, specifies the nonhuman vertebrate to be a dog. Support for this claim can be found on page 21, line 20.

Claim 75 is directed to a method for treating or preventing anemia in a nonhuman vertebrate. Support for this claim can be found in the specification at least on page 47.

Claims 76 and 77 which depend from claim 75 relate to the instance when the nonhuman vertebrate is a horse and the CpG oligonucleotide is administered before or after a race, respectively. Support is found on page 47.

Rejection of Claims 12, 41, 50, and 63 under 35 U.S.C. § 112 ¶ 2

The Examiner rejected claims 12, 41, 50, and 63 for indefiniteness due to lack of proper antecedent basis. As pointed out by the Examiner, these claims as originally presented depend from claims 1, 27, 42, and 51, respectively, none of which contains the required X_1X_2 and X_3X_4 language. Applicant hereby amends claims 12, 41, 50, and 63 to depend from claims 11, 40, 49, and 62, respectively, each of which does contain the required X_1X_2 and X_3X_4 language. Support for the amended claims can be found in the specification at pages 12, line 30, through page 13, line 6; and at page 36, lines 26-30.

Applicant respectfully requests the Examiner to withdraw the rejection of claims 12, 41, 50, and 63 as amended because the claims now have proper antecedent basis and are supported in the specification.

Rejection of Claims 1-65 under 35 U.S.C. § 112 ¶ 1

The Examiner rejected claims 1-65 under 35 U.S.C. § 112 ¶ 1 as lacking enablement due to the Examiner's perceived need for an undue amount of experimentation to make and use the invention as claimed. In support of her argument, the Examiner cites three references: Zhao Q et al., Effect of different chemically modified oligodeoxynucleotides on immune stimulation, *Biochem Pharmacol* 51:173-82 (1995); Crystal RG, Transfer of genes to humans: early lessons and obstacles to success, *Science* 270:404-10 (1995); and Jones TR et al., Synthetic oligodeoxynucleotides containing CpG motifs enhance immunogenicity of a peptide malaria vaccine in *Aotus* monkeys, *Vaccine* 17:3065-71 (1999).

Applicants respectfully traverse the Examiner's rejection of claims 1-65 under 35 U.S.C. § 112 ¶ 1 for the following reasons. First, the Examiner appears to have based the rejection on the ground that the application does not teach one skilled in the art how to make and use the *optimal* oligonucleotide. However, enablement of the invention is independent of making and using the *optimal* oligonucleotide. The specification teaches that CpG oligonucleotides, as defined by the presence of an unmethylated CpG dinucleotide and a minimum length of at least 8 nucleotides, are useful for the invention. *Optimization* of a particular oligonucleotide, though possibly desirable, is not required to make and to use the invention. Rather, the enablement provision of 35 U.S.C. § 112 ¶ 1 requires only that the specification provide sufficient information to permit one skilled in the art *to make and to use* the invention without the exercise of undue experimentation. This legal burden, we believe, has been amply met by the specification, which teaches specific formulas, specific sequences, and specific methods and modifications to use to make the oligonucleotides of the invention. If anything, the specification supplies more instruction and direction than is required by the law.

Second, Applicants respectfully differ with the Examiner's assertion that the practice of the invention would require an undue amount of experimentation. The broadest claims recite methods based on administration of a CpG oligonucleotide and the oligonucleotide is at least 8 nucleotides long. According to the invention, this is sufficient to make and use the invention. Even if one were improperly to apply the higher standard of optimization, i.e., something beyond the legal standard for enablement, in order to determine what is enabled by the specification, only a minimum amount of experimentation is called for. As defined in the specification, a CpG oligonucleotide has a sequence including at least the formula $5'X_1CGX_23'$, wherein X_1 and X_2 are nucleotides. Of the at least 8 nucleotides of the CpG oligonucleotide, the at least four other nucleotides outside the four embraced by the formula 5' X_1CGX_2 3' are there for efficient cellular uptake, as disclosed in the specification at page 36, lines 30-31. As such, the identities of these at least four other nucleotides are, from the point of view of enablement, irrelevant and need not enter into consideration of what constitutes undue experimentation. The issue then turns on the formula 5' X_1CGX_2 3', which lends itself to a total of only 16 possible sequences 5' X_1CGX_2 3' when X_1 and X_2 are selected from the usual deoxynucleotides G, C, A, and T. Those skilled in

the art would agree that making and using 16 oligonucleotides is certainly not out of the ordinary or excessive. Such a minimal amount of experimentation is in stark contrast to that degree held by the CAFC to be so excessive as to be not enabling:

> [We] do not intend to imply that generic claims to genetic sequences cannot be valid where they are of a scope appropriate to the invention disclosed by an applicant. That is not the case here, where Amgen has claimed every possible analog of a gene containing about 4,000 nucleotides, with a disclosure only of how to make EPO and a very few analogs.

Amgen, Inc. v. Chugai Pharmaceutical Co., Ltd., 927 F.2d 1200, 1214 (1991). In the instant invention, provided all 16 sequences were made and at least one of the 16 were tested and found useful, a person skilled in the art would be able to practice the invention. No doubt further experimentation could be undertaken, directed to a rather limited number of modifications involving either the backbone or the bases, to find an *optimal* oligonucleotide for the particular application. However, practice of the invention does not necessarily require optimization, so such further experimentation is not required to practice the invention. Therefore, even when viewed from the Examiner's point of view, the broadest claims require no more than a reasonable amount of experimentation to practice the claimed invention.

Third, in addition to the minimal experimentation requirements outlined above, the invention as a whole provides further guidance to the skilled artisan by disclosing particular oligonucleotides and formulas. For example, the specification at page 12, lines 27-29 and at page 36, lines 21-25 discloses CpG oligonucleotides having a sequence including at least the formula 5' $X_1X_2CGX_3X_4$ 3' wherein X_1X_2 are nucleotides selected from the group consisting of: GpT, GpG, GpA and ApA, and X_3X_4 are nucleotides selected from the group consisting of: TpT, CpT and GpT. This formula encompasses only 12 possible sequences, as does the formula 5' $TCNTX_1X_2CGX_3X_4$ 3' wherein N is either zero or one nucleotide and X_1X_2 and X_3X_4 are as just described. Furthermore, the specification discloses specific sequences to guide the person of ordinary skill in the art. Together, these features provide much more guidance and less randomness than appears to be implied by the Examiner's statement, "The quantity of experimentation necessary to practice the invention would require de novo determination of the claimed treatment effects *for any random sequence* claimed ..." [Emphasis added.]

Fourth, with respect to the specific statements in the Zhao reference cited by the Examiner, Applicant respectfully points out that the fact that "stimulatory effects are dependent upon particular sequences of the oligonucleotide but independent of whether the oligonucleotide is antisense, sense, or scrambled with respect to their target sequences" in fact serves to support the argument that the amount of experimentation is limited. This is so because the statement teaches that it is unnecessary to try randomly (or even non-randomly) selected sense and antisense sequences directed to any of an immense number of possible candidate target genes. Rather, it is necessary only to identify particular sequences containing an unmethylated CpG dinucleotide, e.g., selected from among the 16 possible sequences embraced by the formula 5' X_1CGX_2 3'.

The quoted teaching from Krieg et al. that "*optimal* B cell activation requires a DNA motif in which an unmethylated CpG dinucleotide is flanked by two 5' purines and two 3' pyrimidines" [emphasis added] deals again with *optimization*, and says nothing with regard to enablement of the instant invention. Even from an optimization standpoint, the statement again serves to narrow rather than expand the amount of experimentation. Without meaning to be bound to the above teaching in Krieg, at the very least, applying this teaching to the formula 5' X_1CGX_2 3' suggests only four possible preferred sequences to try, i.e., X_1 is A or G and X_2 is C or T. Significantly, the quotation from Krieg et al. does not contradict the teaching of the instant invention because, just as to specify what is optimal does not equate optimal with functional, neither does it equate non-optimal with non-functional.

Furthermore, while it may be true that Xhao teaches that the "degree of substitution with thioate linkages in the oligonucleotide can influence the stimulatory activity of the oligonucleotides," this statement is again directed to *optimization* and has no bearing on what is enabled by the specification. The teaching is fully disclosed by the specification anyway. What the Xhao reference also specifically teaches is that "phosphorothioate oligodeoxynucleotides [display] a greater stimulatory effect than partially modified phosphorothioate oligonucleotides" both in vitro and in vivo. In other words, those skilled in the art would likely rely on phosphorothioate oligonucleotides and focus on selecting the sequence rather than the backbone. This again suggests the attention of the skilled artisan would be directed to a reasonably limited

number of variables and thus supports the conclusion that no more than a reasonable amount of experimentation is needed to make and use the invention.

Turning to points from other references cited by the Examiner, the difference between mice and other whole organisms, as it pertains to the enablement issue, is not as significant as the Examiner seems to suggest. First, the adage that "humans are not simply large mice" for treatment in gene therapy, simply has no bearing on enablement. No one can dispute the fact that positive results from preclinical studies in mice and other small mammals provide the basis for countless clinical trials, peer-reviewed medical and scientific publications, and patents. The accompanying passage from the Crystal reference is directed largely to problems related to toxicity of gene therapy vectors when applied to humans. Without meaning to make any assertion about toxicity with regard to the present invention, toxicity represents a clinical problem distinct from the legal requirement of enablement. Lack of toxicity is not a requirement of enablement. In addition, as specified at page 21, lines 19-21, a subject can be a "human or vertebrate animal including but not limited to a dog, cat, horse, cow, pig, sheep, goat, chicken, primate, e.g., monkey, fish (aquaculture species), e.g., salmon, rat, and mouse."

Second, the reference by Jones et al., the quoted statements from the introduction to that paper notwithstanding, shows in Table 1 that two of three oligonucleotides tested were effective in at least two primate species. Furthermore, the Jones reference goes on to show how easy it is to find good oligonucleotides for human use simply by screening a panel of candidate CpG oligonucleotides using human peripheral blood mononuclear cells in vitro. This too seems to pose no more than a reasonable burden on one skilled in the art to practice the invention. In addition, it is unclear what significance to attach to the cited (unpublished) observation concerning species specificity that "While murine immune cells respond to a wide variety of CpG motifs, cells obtained from humans and other primates respond to a much more restricted subset." The fact that human cells respond to a restricted subset of CpG oligonucleotides compared to those effective in mice does not necessarily pose a problem in terms of enablement.

Applicant is unsure what the Examiner intends to convey in pointing out the fact that mice, unlike humans, display basal hematopoietic activity in the spleen. In mice the spleen is effectively a readily accessible counterpart to bone marrow, whereas in larger vertebrates like

humans, the marrow space is large enough to accommodate the normal requirements of hematopoiesis.

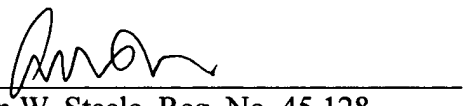
In view of the foregoing arguments, Applicant respectfully requests the Examiner to withdraw the objection to claims 1-65 made on the basis of 35 U.S.C. § 112 ¶ 1.

SUMMARY

Claims 12, 41, 50, and 63 are amended to furnish them with proper antecedent basis and thus to overcome the objection on the basis of 35 U.S.C. § 112 ¶ 2. Applicant traverses the Examiner's rejection of claims 1-65 made on the basis of 35 U.S.C. § 112 ¶ 1, for the reasons given above outlining the actual amount of experimentation reasonably associated with the invention as claimed. The specification is also amended to correct deficiencies in the assignment of SEQ ID NOs to specific nucleic acid sequences which appear in a sequence listing previously submitted to the Patent and Trademark Office. New claims 66-77 are presented and supported by the specification.

It is believed the claims are now in condition for allowance. Favorable action is earnestly solicited. If for any reason the Examiner has any question or would require further information, he is encouraged to contact the Applicant's representative at the number presented below.

Respectfully submitted,



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